UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/734,936	12/12/2003	Wonchul Suh	CL1878USNA	2510

23906 7590 10/04/2007

E I DU PONT DE NEMOURS AND COMPANY
LEGAL PATENT RECORDS CENTER
BARLEY MILL PLAZA 25/1128
4417 LANCASTER PIKE
WILMINGTON, DE 19805

EXAMINER	
MITCHELL, LAURA MCGILLEM	
ART UNIT	**PAPER NUMBER**
1636	

NOTIFICATION DATE	DELIVERY MODE
10/04/2007	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PTO-Legal.PRC@usa.dupont.com

Office Action Summary	Application No.	Applicant(s)	
	10/734,936	SUH, WONCHUL	
	Examiner	Art Unit	
	Laura M. Mitchell	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 July 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,4,7-11,13-17,20 and 26-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4,7-11,13-17,20 and 26-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 December 2003* is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/11/2007 has been entered.

It is noted that claims 1 and 17 have been amended, claims 2, 5-6, 12, 18-19 and 21-25 have been cancelled in the response filed 7/11/2007. It is noted that previously claims 5-6, 23-24 and 30 (now cancelled) were previously indicated as allowable if written in independent form. However, on further consideration, new art has been applied to the claims now incorporating the limitations of claims 5-6 and 23-24 and 30. Claims 1, 3-4, 7-11, 13-17, 20, 26-and 30 are under examination.

Claim Objections

Claim 1 is objected to because of the following informalities: Claim 1 has been amended to recite in step b) (i) the phrase "X is an expressible DNA fragment having homology to the second recombination **element**" where previously the phrase read "second recombination **region**". However, it appears that X is part of the "one second recombination element" in step b). Therefore X would be homologous to itself and the recited step is now grammatically incorrect. The recited step appears to make more

sense when it read "second recombination **region**" because the previous step a)(iv) recites a "second recombination **region**". Appropriate correction is required.

Claim 3 is objected to because of the following informalities: claim 3 recites the phrase "wherein **either** the first expressible DNA fragment" but does not recite an alternative fragment. Claim 3 was previously drawn to a method comprising a "first or second expressible DNA fragment". Since the claim has been amended to remove the second expressible DNA fragment, the word "either" renders the claim grammatically incorrect. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 30 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 30 is vague and indefinite because it has been amended to recite the phrase "Claims 31, or 17" and it appears that the claim has been amended to be dependent on claim 31, which is not present in claims 1-30, so it is not clear on which claims that claim 30 is dependent. The claim could be interpreted that the dependency of the claim has been altered from claims 1 or 17 to claims 3 or 17. If so, the number 1 should have been indicated as removed by double bracketing (e.g. [[1]], or 17). The

claims could also be interpreted as if claim 30 is dependent on claims 1, 3 or 17. For examination, the claim will be interpreted as if it is dependent on claims 3 or 17.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Applicant's arguments, see REMARKS, filed 7/11/2007, with respect to the rejection(s) of claim(s) 1, 3, 7-11, 15-16 under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (of record) in view of Yu et al (of record) and further in view of Prideaux et al (of record) have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of newly found prior art (see below).

Applicant's arguments, see REMARKS, filed 7/11/2007, with respect to the rejection(s) of claim(s) 1, 3-4, 7-8, 11, 13-17, 20-22 and 26-29 under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (of record) in view of Yu et al (of record) and further in view of Welch et al (of record) as evidenced by Guzman et al have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of a newly found prior art reference (see below).

Claims 1, 3, 7, 10-11 and 15-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (of record) in view of Yu et al (of record), in view of Prideaux et al (of record) and further in view of Chen et al (U.S. Patent App. Pub. No. 2004/0043003, filed 10/25/2002).

The method of claim 1 has been amended to include the limitation that the first chromosomal region on the bacterial chromosome has homology to the first recombination region and wherein the first chromosomal region is either upstream of a bacterial promoter or an inter-operon chromosomal integration site.

The teaching of Perkins et al, Yu et al (of record) and Prideaux et al have been detailed in a previous Office action, mailed 5/25/2006. Specifically, Prideaux et al teach a method of producing a toxin for use as a vaccine in a modified microorganism such as *E.coli* (see column 2, lines 46-50 and column 3, lines 22-26, in particular). Prideaux et al teach that that it is useful to produce a gene that has been inactivated by introducing a targeting construct introduced by site-specific homologous recombination. Prideaux et al teach that it may be undesirable to have a functional antibiotic resistance gene incorporated into the modified microorganism. Prideaux et al discloses a targeting construct which includes genetic elements, such as site specific recombination sites, which facilitate excision of the antibiotic resistance gene once the targeting construct has undergone homologous recombination with the host chromosome (see column 3, lines 41-62, column 6, lines 48-56 and column 18, lines 11-20, in particular). Prideaux et al teach that the vectors can be integrated into the host chromosome wherein the genes are expressed (see column 7, lines 20-30, for example). Perkins et al in view of Yu et al

and in view of Prideaux et al render obvious a method for the directed integration of an expressible DNA marker. However, Perkins et al in view of Yu et al and in view of Prideaux et al do not specifically teach that the host cell chromosome region comprises a first chromosomal region that is either upstream of a bacterial promoter or an inter-operon chromosomal integration site.

Chen et al teach methods and clinical grade vectors for delivery of a gene of interest (i.e. a therapeutic gene) to a host (see paragraph 0024). Chen et al teach that non-pathogenic microorganisms can be used for delivery, including bacteria (see paragraph 0055-0056, for example). Chen et al teaches vectors comprising gene sequences encoding therapeutic polypeptides (see abstract) but without antibacterial selection markers (see paragraph 0015 and 0062, for example). Chen et al teach that gene of interest may or may not include a promoter (see paragraph 0041, for example). Chen et al teach that expression cassettes can be integrated into bacterial chromosomes (see paragraph 0065). Chen et al teach that chromosomal integration can be mediated by sequences that have homology to flanking sequences of a target site on the chromosome (see paragraph 0068). Absent evidence to the contrary, a host bacterial cell promoter would be in the upstream flanking sequence of a gene of interest. Chen et al exemplify an expression cassette comprising a promoter. Chen et al exemplify sequences that are homologous to a HO gene which will facilitate chromosomal integration of the expression cassette at the HO locus (see paragraph 0112 and Figure 6B). Chen et al illustrate a recombination scheme wherein the "HO left" is the 5' end of the HO promoter (Figure 6), which meets the limitation of a bacterial

chromosome wherein a region is upstream of a bacterial promoter. Chen et al illustrate that after recombination the HO promoter has been replaced with a heterologous promoter operably linked to a gene of interest. Therefore, Chen et al teach an integration of a sequence at a chromosomal site that is upstream of a promoter.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method rendered obvious by Perkins et al in view of Yu et al in view of Prideaux et al and integrate an expressible DNA fragment at a first chromosomal region upstream of a bacterial promoter because Prideaux et al suggests that vectors can be integrated into the host chromosome wherein the genes are expressed and Chen et al demonstrates a recombination method wherein the first recombination sequence is at the 5' end of a HO promoter. The motivation to target a chromosomal region upstream of a promoter would be the expected benefit of being able to remove an endogenous promoter and replace it with a promoter of choice as exemplified by Chen et al. The skilled artisan would realize that inducible promoters could be integrated near the gene of interest to add transcriptional control. There is a reasonable expectation of success because such methods have worked in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Perkins et al in view of Yu et al in view of Prideaux et al and further in view of Chen et al render obvious a method for the directed integration of an expressible DNA fragment lacking a selectable marker into a

chromosome of an *E.coli* wherein the first chromosomal region is upstream of a bacterial promoter (**claim 1**).

Perkins et al teach that target nucleic acids may include one or more genes and/or their associated regulatory regions (see paragraph 0033, in particular), which meets the limitation of a method where the first expressible DNA fragment is selected from the group consisting of regulatory elements, promoters, genes, coding sequence and open reading frames (**claim 3**). Prideaux et al teach that an introduced expressible gene (APX1 A) is under the control of a regulatable promoter/operator element consisting of the *E. coli* phage T5 promoter and two lac operator sequences, which meets the limitation of a method wherein the expressible DNA is a promoter selected from a group of bacterial and phage promoters (**claim 7**) and wherein the promoter is a T5 promoter (**claim 10**).

Prideaux et al teach that it may be undesirable to have a functional antibiotic resistance gene incorporated into the modified microorganism. Prideaux et al discloses a targeting construct which includes genetic elements, such as repeat sequences or site specific recombination sites, which facilitate excision of an antibiotic resistance gene once the targeting construct has undergone homologous recombination with the host chromosome (see column 3, lines 41-62, column 6, lines 48-56 and column 18, lines 11-20, in particular), which meets the limitation of a method wherein the selectable marker is an antibiotic resistance marker (**claim 11**).

Perkins et al teach that the first and second elements can be produced using PCR amplification (see paragraph 0011, in particular), which meets the limitation of a method wherein the recombination elements are generated by PCR (**claim 15**). Perkins et al also teach that the sequence specific recombination regions can be at least about 15 nucleotides and an especially preferred size is from about 25 to about 60 nucleotides (see paragraphs 0023 and 0032, in particular), which meets the limitation of a method wherein recombination elements are from about 25 bases to about 4000 bases (**claim 16**).

Claims 1, 3-4, 7-11 and 13-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (of record) in view of Yu et al (of record) in view of Welch et al (of record) as evidenced by Guzman, et al (of record) and in view of Chen et al (U.S. Patent App. Pub. No. 2004/0043003, filed 10/25/2002), and further in view of Reim et al (U.S Patent No. 5,122,457, 6/16/1992).

Applicants claim a method for the directed integration of an expressible DNA fragment lacking a selectable marker into a chromosome of an *E.coli* wherein the first chromosomal region is upstream of a bacterial promoter, and wherein the expressible DNA fragment is a promoter comprising positive and negative regulatory sites. Applicants further claim the method wherein the recombination sites are selected from the group of lox, frt, dif and att and the corresponding recombinases are used.

The teachings of Perkins et al (of record) in view of Yu et al (of record) and further in view of Welch et al (of record) as evidenced by Guzman et al (of record) have

been detailed in previous Office action mailed 5/25/2006. Perkins et al in view of Yu et al and further in view of Welch et al as evidenced by Guzman et al render obvious a method for the directed integration of an expressible DNA fragment lacking a selectable marker into a chromosome of an *E.coli.* However, Perkins et al in view of Yu et al and further in view of Welch et al as evidenced by Guzman et al do not specifically teach a method wherein the first chromosomal region is upstream of a bacterial promoter.

The teachings of Chen et al are detailed in the rejection above. Specifically, Chen et al exemplify an expression cassette comprising a promoter. Chen et al illustrate a recombination scheme wherein the "HO left" is the 5' end of the HO promoter (Figure 6), which meets the limitation of a bacterial chromosome wherein a region is upstream of a bacterial promoter. Chen et al illustrate that after recombination the HO promoter has been replaced with a heterologous promoter operably linked to a gene of interest. Therefore, Chen et al teach an integration of a sequence at a chromosomal site that is upstream of a promoter.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method rendered obvious by Perkins et al in view of Yu et al in view of Welch et al as evidenced by Guzman et al and integrate an expressible DNA fragment at a first chromosomal region upstream of a bacterial promoter because Chen et al demonstrates a recombination method wherein the first recombination sequence is at the 5' end of a HO promoter. The motivation to target a chromosomal region upstream of a promoter would be the expected benefit of being able to remove an endogenous promoter and replace it with a promoter of choice as

exemplified by Chen et al. The skilled artisan would realize that inducible promoters could be integrated near the gene of interest to add transcriptional control. There is a reasonable expectation of success because such methods have worked in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Perkins et al in view of Yu et al in view of Welch et al as evidenced by Guzman et al, in view of Chen et al render obvious a method for the directed integration of an expressible DNA fragment lacking a selectable marker into a chromosome of an *E.coli* wherein the first chromosomal region is upstream of a bacterial promoter (**claim 1**).

Claims 7-10 are drawn to a method for the directed integration of a promoter comprising positive and negative regulatory sites for control of a regulatory circuit that comprises a lac operator site and wherein the promoter is a phage T5 promoter, a phage T7 promoter or a lac promoter. Welch et al teach that the λ Red recombinase system and PCR generated recombinant fragments encoding an araBAD promoter was used to replace a native promoter (see paragraph 0063-0064, in particular). Welch et al cite Guzman et al as a reference regarding the araBAD promoter. Guzman et al teach that pBAD vectors (pBAD 18 and pBAD33) comprising a promoter and a gene comprising **positive and negative regulators** of the promoter (see page 4121, abstract and page 4122, Figure 1, for example) which reads on a promoter that is a bacterial or phage promoter and comprises positive and negative regulatory sites (**claim 7-8**).

However, Perkins et al in view of Yu et al and further in view of Welch et al do not teach a method wherein the expressible DNA is a promoter comprising regulatory sites for a lac operator and wherein the promoter is a phage T5 promoter, a phage T7 promoter or a lac promoter. Reim et al teach expression systems using bacteriophage T7 promoter regions to produce large quantities of polypeptides. Reim et al disclose that a variety of promoter sequences are known in the expression of foreign or heterologous genes in *E.coli*, including the inducible promoter lac. Reim et al teach that T7 promoters are of particular interest because there is a strong interaction between bacteriophage T7 promoters and T7 RNA polymerase (see column 2, lines 20-65, for example). Reim et al teach that T7 promoter can be linked in a correct reading frame with a gene encoding a desired heterologous protein. Reim et al teach a preferred embodiment in which the T7 promoter is a strong promoter identified as class III (see column 4, lines 1-11 and claim 6, lines 39-61, for example).

It would have been obvious to one of skill in the art at the time the invention was made to modify the method made obvious by Perkins et al in view of Yu et al and further in view of Welch et al as evidenced by Guzman et al and incorporate a T7 promoter in the place of the araBAD promoter as taught by Welch because Reim et al teach that T7 promoter can be linked in a correct reading frame with a gene encoding a desired heterologous protein. The motivation to replace an araBAD promoter with a T7 promoter would be the expected benefit of being able to use a strong promoter as is disclosed by Reim et al to induce polypeptide synthesis. There is a reasonable expectation of success to use a T7 promoter for integration into a chromosome because T7 promoters

are known in the prior art and T7 promoters have worked previously in the cited reference. **Therefore Perkins et al in view of Yu et al in view of Welch et al as evidenced by Guzman et al and further in view of Reim et al render obvious a method wherein the expressible DNA is a promoter comprising regulatory sites for a lac operator and wherein the promoter is a phage T7 promoter or a lac promoter (claims 9-10).**

Perkins et al teach that target nucleic acids may include one or more genes and/or their associated regulatory regions (see paragraph 0033, in particular), which meets the limitation of a method where the first expressible DNA fragment is selected from the group consisting of regulatory elements, promoters, genes, coding sequence and open reading frames (**claim 3**).

Welch et al teach an embodiment of a method that uses the λ Red recombinase system. Welch et al further teach that the PCR generated nucleotide sequence was introduced into a cell carrying a λRed expression plasmid (see paragraph 0049, in particular), which meets the limitation of a method wherein the site-specific recombinase is expressed by a gene residing on a plasmid (**claim 4**). Welch et al also teach incorporation of an antibiotic resistance gene flanked by FLP resistance target sites, which meets the limitations of a method wherein the selectable marker is an antibiotic resistance marker (**claim 11**). Welch et al teach that the resistance plasmid was excised by introduction of a helper FLP plasmid that acts on the FLP recognition target sites (see paragraph 0049, in particular) which meets the limitations of a method wherein the site specific recombinase is FLP recognizing the *frt* recombination site (**claims 13-14**).

Claims 17, 20 and 26-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (of record) in view of Yu et al (of record) in view of Welch et al (of record) and further in view of Reim et al (U.S Patent No. 5,122,457, 6/16/1992). This is a NEW rejection.

Applicants claim a method for the integration of a foreign promoter in place of a bacterial chromosome promoter in a recombination efficient *E.coli* host cell, wherein the foreign promoter is selected from the group consisting of phage T5 promoter, a phage T7 promoter or a lac promoter.

The teachings of Perkins et al (of record) in view of Yu et al (of record) and further in view of Welch et al (of record) have been detailed in previous Office action mailed 5/25/2006. Perkins et al (of record) in view of Yu et al (of record) and further in view of Welch et al (of record) render obvious a method for the integration of a foreign promoter in place of a bacterial chromosome promoter in a recombination efficient *E.coli* host cell.

Specifically, Welch et al teach a method of inserting genes into a bacterial chromosome to construct constitutive promoter mutants in *E.coli* (see paragraph 0029). Welch et al teach that the λ Red recombinase system and PCR generated recombinant fragments encoding an araBAD promoter (i.e. a foreign promoter) were used to replace a native promoter in order to control transcription of a dsdC gene (see paragraph 0063-0064, in particular). Welch et al suggest that other suitable replacement promoters may be used, including Class III- regulated promoter or inducible promoters (see paragraph 0064, in particular). Perkins et al (of record) in view of Yu et al (of record) and further in

view of Welch et al (of record) **do not specifically teach incorporation** of a phage T5 promoter, a phage T7 promoter or a lac promoter.

The teaching of Reim et al has been detailed in the above rejection. Specifically, Reim et al teach that T7 promoters are of particular interest because there is a strong interaction between bacteriophage T7 promoters and T7 RNA polymerase (see column 2, lines 20-65, for example).

It would have been obvious to one of skill in the art at the time the invention was made to modify the method made obvious by Perkins et al in view of Yu et al and further in view of Welch et al and incorporate a T7 promoter in the place of the araBAD promoter taught by Welch because Reim et al teach that T7 promoter can be linked in a correct reading frame with a gene encoding a desired heterologous protein. The motivation to replace an araBAD promoter with a T7 promoter would be the expect benefit of being able to use a strong promoter as is disclosed by Reim et al to induce polypeptide synthesis. There is a reasonable expectation of success to use a T7 promoter for integration into a chromosome because T7 promoters are known in the prior art and T7 promoters have worked previously in the cited reference. Therefore, Perkins et al (of record) in view of Yu et al (of record) in view of Welch et al (of record) and further in view of Reim et al render obvious a method for the integration of a foreign promoter in place of a bacterial chromosome promoter in a recombination efficient *E.coli* host cell, wherein the foreign promoter is a phage T7or a lac promoter (**claim 17**).

Welch et al teach an embodiment of a method that uses the λ Red recombinase system. Welch et al further teach that the PCR generated nucleotide sequence was

introduced into a cell carrying a Red expression plasmid (see paragraph 0049, in particular), which meets the limitation of a method wherein the site-specific recombinase is expressed by a gene residing on a plasmid (**claim 20**). Welch et al also teach that the resistance plasmid was excised by introduction of a helper FLP plasmid that acts on the FLP recognition target sites (see paragraph 0049, in particular), which meets the limitation of a method wherein the site specific recombinase is FLP recognizing the *frt* recombination site (**claims 26-27**). Perkins et al teach that the first and second homologous recombination sequences of the method can comprise selectable markers and a promoter. Perkins et al also teach that the first and second elements can be produced using PCR amplification (see paragraph 0011, in particular), which meets the limitation of a method wherein the recombination elements are generated by PCR (**claim 28**). Welch et al teach an embodiment of their method that uses the λ Red recombinase system and comprising PCR generation of 36-50 nucleotide extensions that are homologous to a chromosomal region adjacent to the gene of interest (see paragraph 0049, in particular), which meets the limitation of a method wherein the recombination elements are from about 25 bases to about 4000 bases (**claim 29**).

Claim 30 is drawn to the method according to claim 17 wherein steps (d)-(f) are repeated one or more times to insert a foreign promoter into a bacterial chromosome in place of the bacterial promoter. Steps (d)-(f) comprise transforming the host cells with the first and second recombination elements, selecting and isolating transformed hosts and subsequently expressing a site-specific recombinase to excise the selectable marker. It would be obvious to the skilled artisan to repeat steps (d)-(f) of the method

rendered obviated by Perkins et al in view of Yu et al, in view of Welch et al and further in view of Reim et al to integrate a foreign promoter into a bacterial chromosome in place of the bacterial promoter in order to generate the desired yield of cells with engineered chromosomes comprising foreign promoters. The motivation to repeat transformation, selection and isolation steps followed by excision of a undesired selectable marker would be the expected benefit of being able to perform the method until the desired host cells are produced (**claim 30**).

Claims 17, 20 and 26-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (of record) in view of Yu et al (of record) in view of Welch et al (of record) in view of Reim et al (U.S Patent No. 5,122,457, 6/16/1992) and further in view of Bryan et al (U.S Patent No. 6,436,682, 8/20/2002).

Applicants claim a method for the integration of a foreign promoter in place of a bacterial chromosome promoter in a recombination efficient *E.coli* host cell, wherein the foreign promoter is selected from the group consisting of phage T5 promoter, a phage T7 promoter or a lac promoter.

The teaching of Perkins et al in view of Yu et al in view of Welch et al and further in view of Reim et al (U.S. Patent No. 5,122,457) have been detailed above. Perkins et al in view of Yu et al in view of Welch et al and further in view of Reim et al (U.S. Patent No. 5,122,457) render obvious a method for the integration of a foreign promoter in place of a bacterial chromosome promoter in a recombination efficient *E.coli* host cell, wherein the promoter is a T7 promoter and a lac promoter. Perkins et al in view of Yu et

al in view of Welch et al and further in view of Reim et al (U.S. Patent No. 5,122,457) do not specifically teach the promoter as a phage T5 promoter.

Bryan et al teach methods of producing fluorescent proteins such as luciferase from nucleic acid expression constructs. Bryan et al teach that preferred inducible promoters for use in *E.coli* are the T7 phage promoter or T7-like phage promoters such as T5 phage promoter or lac promoters (see column 52, lines 31-55, column 58, lines 16-25 or column 59, lines 35-47, for example).

It would be obvious to modify the method rendered obvious by Perkins et al in view of Yu et al in view of Welch et al and further in view of Reim et al and use a phage T5 promoter or a lac promoter as taught by Bryan et al in place of a T7 promoter or a BAD promoter because the claimed promoters were well known in the prior art and the substitution of one known element (i.e. phage T5 promoter or a lac promoter) for another (i.e. BAD promoter or T7 promoter) would yield predictable results to the skilled artisan at the time the invention was made. It is predictable that the method for integration of a foreign promoter would work with a phage T5 promoter or a lac promoter because they are promoters well known in the art. There is a reasonable expectation of success to use a T5 or lac promoter for integration into a chromosome because they are known in the prior art. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Perkins et al (of record) in view of Yu et al (of record) in view of Welch et al (of record) in view of Reim et al and

further in view of Bryan et al render obvious a method for the integration of a foreign promoter in place of a bacterial chromosome promoter in a recombination efficient *E.coli* host cell, wherein the foreign promoter is a phage T5 or T7 promoter or a lac promoter (**claim 17**).

Welch et al further teach that the PCR generated nucleotide sequence was introduced into a cell carrying a λRed expression plasmid (see paragraph 0049, in particular), which meets the limitation of a method wherein the site-specific recombinase is expressed by a gene residing on a plasmid (**claim 20**). Welch et al also teach that the resistance plasmid was excised by introduction of a helper FLP plasmid that acts on the FLP recognition target sites (see paragraph 0049, in particular), which meets the limitation of a method wherein the site specific recombinase is FLP recognizing the *frt* recombination site (**claims 26-27**). Perkins et al also teach that the first and second elements can be produced using PCR amplification (see paragraph 0011, in particular), which meets the limitation of a method wherein the recombination elements are generated by PCR (**claim 28**). Welch et al teach an embodiment of their method that comprises PCR generation of 36-50 nucleotide extensions that are homologous to a chromosomal region adjacent to the gene of interest (see paragraph 0049, in particular), which meets the limitation of a method wherein the recombination elements are from about 25 bases to about 4000 bases (**claim 29**).

Claim 30 is drawn to the method according to claim 17 wherein steps (d)-(f) are repeated one or more times to insert a foreign promoter into a bacterial chromosome in place of the bacterial promoter. Steps (d)-(f) comprise transforming he host cells with

the first and second recombination elements, selecting and isolating transformed hosts and subsequently expressing a site-specific recombinase to excise the selectable marker. It would be obvious to the skilled artisan to repeat steps (d)-(f) of the method rendered obviated by Perkins et al in view of Yu et al, in view of Welch et al and further in view of Reim et al to integrate a foreign promoter into a bacterial chromosome in place of the bacterial promoter in order to generate the desired yield of cells with engineered chromosomes comprising foreign promoters. The motivation to repeating transformation, selection and isolation steps followed by excision of a undesired selectable marker would be the expected benefit of being able to perform the method until the desired host cells are produced (**claim 30**).

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura M. Mitchell whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Laura McGillem Mitchell, PhD
Examiner
9/21/2007

CELINE QIAN, PH.D.
PRIMARY EXAMINER